January 26, 1950.

Dear Max:

Enclosed is H-226, which may be one of the most interesting diploid cultures yet encountered. Unlike the other cultures you've received, this one is derived from a cross of "standard" parents, not carrying Het [asxfar as I know]. Lac_4- B-M- (W-67) x W-1262 (TLB_1 Lac_1 Mal Gal Xyl Mtl Ar Stl V_1^r) Diploid prototrophs in such crosses are extremely rare, but can be picked out with moderate trouble because Lac_1 and Lac_4 are extremely closely linked, and most of the Lac+ prototrophs, which can be picked out by inspection on EMS agar, turn out to be diploid double heterozygotes rather than haploid recombinants. In this series, H-226 was the sole Lac+ prototroph found among several thousand inspected from several repetitions of the cross (unfortunately, the fertility is rather low).

At any rate, H-226 is unique insofar as it seems to be heterozygous for most if not all of the factors in which the parents differed, including Mal which is here the same factor as is almost invariably hemizygous (H-213 being the only other exception among several hundred diploids, and being obtained only by persistent testing of several hundred Mal+ prototrophs). For this reason, it may be the most likely candidate for obtaining complementary, viable, segregants, as so far at least, there is no sign that it is heterozygous for deficiency. Unfortunately, segregation seems to be somewhat less frequent in these "spontaneous" heterozygotes than in the other diploids, and it may be more difficult to obtain adequate numbers. However, I am sending it posthaste because I think its analysis should take priority over any of the others. With this culture, it might even be a worthwhile gamble to take the culture, as is, for starting material, as there is a good chance that most of the culture is still diploid.

One of my dishwashers is down with the flu, and the others are all troubled with final exams, so I have not been able to get those vials ready for you. Will do so as soon as possible.

Some final dope on previous segregations: 22-75 and 21-251 are both definitely mixtures of Lac ± and Lac-, much as 12-196 and 12-198 were predominantly Lac+. They certainly look to be significantly different from the typical Lac± cultures, but I don't see how we can do much with their interpretation, except possibly with very early platings from your isolated microcolonies, and even then, all we have is a hint of an additional segregation. 2-223 might be more interesting. 5-224, its segregant sib, was Lac-Mal-Vl^s M-. The + colonies isolated early from 5-223 were all alike, and $+^r$ TLB_1, or precisely complementary. However, since these are the two most common types of segregant, I don't know whether one can argue more than coincidence from this

isolated example. But certainly, this is something we have to look out for.

I am very puzzled by the several cases, e.g. 6-88 and 6-25 which were pure on the primary plating, but turn out to be Lacy. Perhaps I don't understand the mechanics of the situation well enough.

Stl has turned out to be in the region of Mal, and it is doubtful therefore whether anything better marked than H-168 is going to turn up. Therefore, I think that this had better be retained as the type for Het diploids.

Have you had any chance to do any writing? Or should I ask whether you've had any time to work at your job with all this extra-curricular activity?

Sincerely,

Joshua Lederberg.